The Weight of Evidence Does Not Support the NTP Draft Styrene Profile's Conclusion of "Reasonably Anticipated to be a Human Carcinogen"

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1 Introduction

The *National Toxicology Program (NTP) 12th Report on Carcinogens Draft Styrene Profile* (NTP, 2009) states: "Styrene is *reasonably anticipated to be a human carcinogen* based on limited evidence of carcinogenicity in humans, sufficient evidence of carcinogenicity in experimental animals, and supporting mechanistic data" (NTP, 2009). Several requirements for "limited" or "sufficient" evidence are not considered, however, and the mode-of-action data do not support human carcinogenicity. As described in more detail below, the effects of styrene observed in experimental animals do not meet the standard of "sufficient evidence in animals" and the underlying mode of action is species-specific and not applicable to humans. In addition, the human data do not consistently show increased mortality or incidence of cancer, and the epidemiologic studies collectively do not support the standard of "limited evidence." Finally, there is no concordance among the human, experimental animal, and mode-of-action data; thus, the styrene data do not meet the criteria for limited evidence of carcinogenicity in humans, sufficient evidence in experimental animals, and supportive mechanistic data. Based on NTP's criteria, styrene should be considered "not classifiable."

2 Several requirements for "limited" or "sufficient" evidence are not considered by NTP

NTP (2006) states the following criteria for "limited" evidence of carcinogenicity in humans: "There is limited evidence of carcinogenicity from studies in humans which indicates that causal interpretation is credible but that alternative explanations, such as chance, bias, or confounding factors could not adequately be excluded." There are no precisely defined standards, however, for when the "limited" criterion is to be deemed satisfied.

It is important to realize that "limited" evidence still requires a positive finding that a causal explanation is credible; it is not simply applied when the data are inconsistent or inconclusive, and the mere presence of some positive evidence in some studies is not by itself grounds to conclude that a causal explanation is credible. Instead, when results are mixed or inconsistent, an evaluation of all of the data must consider whether it is credible to hold that a truly causal relationship exists (and the studies failing to show it do so because of chance and low power or because the true responses are somehow obscured by extraneous factors) or whether it is more credible that there is no causal effect (and the studies

appearing to show an effect of exposure are in fact only showing chance findings or the effects of biases or confounding factors). Making such a judgment requires a thorough and systematic evaluation of the evidence and an evaluation of the relative plausibility of the competing explanations – actual causality partially obscured by chance or bias on the one hand versus bias and confounding creating the spurious appearance of apparent effects on the other. That is, the "limited" evidence category does not simply consist of cases for which there are some positive and some negative results; it is only when a case for a credible (albeit unproven) causative effect can be made that the "limited" evidence characterization should be applied.

In my view, when all the human evidence is evaluated, the low numbers of observed cases and the lack of consistent patterns in cancer outcomes within and across cohorts, combined with concerns about co-exposures and confounding, one comes to the conclusion that a causal relation of styrene exposure and human cancer is not credible, and the standards of "limited" evidence are not met.

NTP (2006) also states the following criteria for "sufficient" evidence of carcinogenicity in experimental animals: "There is an increased incidence of malignant and/or a combination of malignant and benign tumors (1) in multiple species or at multiple tissue sites, or (2) by multiple routes of exposure, or (3) to an unusual degree with regard to incidence, site, or type of tumor, or age at onset."

In this report, I describe the data that show that the NTP criteria for "sufficient" evidence are not met for styrene. In brief, styrene exposure has not been associated with an increased incidence of tumors in multiple species or at multiple tissue sites. There is only evidence for benign or malignant tumors in one species (mice) and one tissue site (lung). Increased tumor incidence was only observed in one inhalation study. Oral gavage studies were equivocal, and tumor incidence was not increased in a study that used intraperitoneal injection as the exposure route. The styrene-related lung tumors in mice were not observed to an unusual degree with regard to incidence, site, tumor type, or age at onset. The tumors were likely a result of cytotoxicity induced by local metabolism of styrene, and this mode of action is supported by mechanistic data as well. The tumors occurred late in life and were not life-shortening. In addition, mouse lung is an organ with a high background incidence of tumors. When all the evidence from experimental animals is evaluated, it is clear that styrene does not fit the NTP standards for "sufficient" evidence.

3 Effects in experimental animals are species-specific and not applicable to humans

The NTP draft profile for styrene states: "There is sufficient evidence for the carcinogenicity of styrene in experimental animals based on the induction of tumors in multiple studies in mice exposed to styrene by two routes of exposure" (NTP, 2009). These tumors are confined only to the lung, and there is inconsistency in the tumor incidence among different strains of mice. Each study that reports lung tumors in mice also suffers from limitations, as described below.

In the only chronic inhalation study of styrene in mice, increased incidences of bronchioalveolar adenomas (benign tumors) were observed in male and female CD-1 mice, but only at the end of the 24-month study period and with no dose-response pattern (Cruzan *et al*., 2001). Females exposed to the highest dose also had an increased incidence (14%) of bronchioalveolar carcinomas (malignant tumors) at the end of the study. The historical control incidences of lung tumors for female CD-1 mice ranged from 0-4% for the laboratory, based on five different studies, and from 0-13.5% for the animal supplier (Charles River), based on nine different studies (Cruzan *et al*., 2001). The adenomas and carcinomas did not differ in tumor morphology between control and treated mice, and histopathologic changes were observed in the terminal bronchioles at all exposure concentrations in a dose-dependent manner at 12-, 18-, and 24-month time points. These changes included decreased eosinophilic staining of Clara cells and epithelial hyperplasia that extended into the alveolar ducts.

In a chronic oral gavage study, male B6C3F1 mice treated with the highest dose of styrene showed an increased incidence (18%) of combined bronchioalveolar adenomas and carcinomas (NCI, 1979). The tumors were only observed at the end of the 21-month study period, and there was no increase in tumor incidence in females in any dose group. No lung tumors were observed in the 20 vehicle controls, even though the historical control incidence for untreated controls at the laboratory ranged from 0-20%. NCI determined that the historical control data were insufficient for vehicle-treated controls. The Draft Background Document (NTP, 2008) examined vehicle controls from two studies in the same laboratory (Litton Bionetics), as well as 14 studies from a nearby laboratory (Hazleton Laboratories), and concluded that the tumor incidence in high dose males in the styrene study could be considered as outside the historical control range. The use of historical controls from a different testing laboratory is not scientifically supported, however. Other NTP investigators have recommended that historical control comparisons should only use controls from the same testing laboratory because of the statistically

significant inter-laboratory variability that has been observed in control mouse lung tumor incidence (Haseman *et al*., 1984). Thus, the new historical control data used by NTP are not valid for assessing the NCI study. Because of the discrepancy in control incidence and the tumor response at only one site and in one sex, NCI concluded that "under the conditions of this bioassay, no convincing evidence for the carcinogenicity of the compound was obtained in…B6C3F1 mice of either sex," and this conclusion should be retained.

In other chronic oral gavage studies, an increased incidence of combined adenomas and carcinomas was observed in the lungs of male and female O_{20} mice (Ponomarkov and Tomatis, 1978). The increased tumor incidence was observed at a dose of styrene associated with the presence of severe lung congestion and early mortality, however, indicating possible styrene toxicity as a complicating factor. Lung tumors were observed in 20/23 (87%) of styrene-treated males and 32/32 (100%) of styrene-treated females, compared to 8/19 (42%) of male and 14/21 (67%) of female vehicle controls, after adjusting for early mortality. Lung tumors were also observed in 34/53 (64%) of male and 25/47 (53%) female untreated controls, indicating that O_{20} mice are very susceptible to the development of lung tumors. The incidence in the styrene-treated males was significantly higher than the vehicle controls only, whereas in styrene-treated females, the incidence was significantly higher than either control group. The authors concluded that "the increased incidence and early appearance of lung tumors could possibly indicate a carcinogenic effect for styrene in O_{20} mice. This experiment, however, has severe limitations, since the dose used was obviously very high, causing severe toxic effects and an early mortality… Results from additional studies are needed before a final evaluation of the carcinogenicity of styrene in rodents can be made." The same group, using a similar study design, reported no increased incidence of tumors of any type in styrene-treated male and female C57Bl mice (Ponomarkov and Tomatis, 1978).

In addition to the inhalation and oral gavage studies in mice, intraperitoneal injection of styrene into A/J mice for seven weeks did not result in an increased incidence of lung tumors at sacrifice 20 weeks later (Brunnemann *et al*., 1992).

There were no increased tumor incidences reported in seven chronic rat studies of styrene by various exposure routes, including inhalation (Cruzan *et al*., 1998; Conti *et al*., 1988; Jersey *et al*., 1978), oral gavage (Conti *et al*., 1988; NCI, 1979; Ponomarkov and Tomatis, 1978), and drinking water (Beliles *et al*., 1985). The NTP draft profile for styrene states: "Data from experimental cancer studies with rats

are insufficient for reaching a conclusion." Thus, there is a lack of generality of the tumorigenic response among rodent species.

In summary, there is evidence that styrene causes an increased incidence of lung tumors in mice after inhalation exposure. Other studies in mice that used oral gavage as the exposure route were equivocal, and tumor incidence was not increased in one study that used intraperitoneal injection. The tumors observed in mice after inhalation of styrene occurred at the end of the chronic study period and were not life-shortening. In addition, the tumors were observed in the presence of lung toxicity. The mouse lung is an organ with a high background incidence of tumors (Cruzan *et al*., 2001). Taken together, the animal data for styrene do not meet the NTP criteria for "sufficient" evidence of carcinogenicity in animals. Neither lung toxicity nor lung tumors have been observed in humans exposed to styrene, and as described below, the proposed mode of action for styrene-induced lung tumors in mice is not applicable to humans, or even to other rodent species.

4 Mode-of-action data demonstrate effects in animals are not applicable to humans

As noted above, styrene induces lung toxicity in mice, but not in rats or humans. In this section, the mode-of-action data that indicate the species-specific origin of this toxicity and how this toxicity is the likely cause of tumor formation in the mouse lung are described.

Inhalation exposures to styrene induce lung toxicity and subsequent tumors in mice and nasal toxicity without tumor development in mice and rats (Cruzan *et al*., 1997; 1998; 2001). Lung toxicity has been reported as decreased cytoplasmic staining and increased replication of Clara cells of the mouse bronchiolar epithelium, cell crowding in the terminal bronchioles, decreased eosinophilia of the mouse bronchiolar epithelium, and hyperplasia of Clara cells in the terminal bronchioles (Cruzan *et al*., 1997; 2001; Green *et al*., 2001a; Roycroft *et al*., 1992). In a chronic inhalation study, Cruzan *et al*. (2001) observed a progression from decreased eosinophilia to hyperplasia of the terminal bronchiolar epithelium, and finally, to hyperplasia that extended into the alveolar ducts. With increasing duration, the exposure concentration at which effects were seen also decreased, such that mice in all dose level groups were affected by the end of the study. Lung toxicity has not been reported in rats or humans exposed to styrene.

The differences in styrene-induced toxicity among mice, rats, and humans can be explained by differences in styrene metabolism. Styrene metabolism occurs mainly in the liver and the lung, and results primarily in the formation of the weakly genotoxic metabolite, styrene-7,8-oxide (SO) (IARC, 2002). SO can be detoxified by glutathione conjugation or by conversion to styrene glycol by microsomal epoxide hydrolase (IARC, 2002). Styrene can also be metabolized by conversion to phenylacetic and phenylaceturic acids (PAA pathway), or by oxidation of its benzene ring, which can lead to the formation of 4-vinylphenol (4-VP) (IARC, 2002). Based on measurements of urinary metabolites, humans metabolize styrene almost exclusively *via* the SO-epoxide hydrolase pathway (Johanson *et al*., 2000). The ring oxidation and PAA pathways play very small roles in styrene metabolism in humans and are used much more in the metabolism of styrene in mice than in rats, indicating that there are major species differences in the overall metabolism of styrene (Johanson *et al*., 2000; Sumner *et al*., 1997).

The formation of SO from styrene is catalyzed by cytochromes P450. CYP2E1 is the predominant enzyme for styrene metabolism in the liver and CYP2F (2F1 in humans, 2f2 in mice, and 2F4 in rats) is the predominant enzyme in the lung (Hynes *et al*., 1999; Wenker *et al*., 2001). There are significant species differences with respect to the activities and concentrations of the CYP2F enzymes in the lung. In mice and rats, the CYP2F enzymes readily metabolize styrene, whereas in humans, CYP2F1 does not appear to metabolize styrene at all (Green *et al*., 2001b). Clara cells are the major cell type in the lung that activates styrene to SO following inhalation exposure, and in mice, these cells are numerous and are spread throughout the airways (Green, 2000). In rats, they are significantly fewer in number, particularly in the terminal bronchiolar region (Green, 2000). In humans, Clara cells are rare and can be found in small numbers in the distal bronchioles (Green, 2000). SO can exist as either an R- or S-enantiomer, with the R-enantiomer being a more potent pneumotoxicant than the S-enantiomer (Gadberry *et al*., 1996). Hynes *et al*. (1999) demonstrated that mouse Clara cells produce five times more total SO and 15 times more R-SO enantiomer than rat Clara cells. Thus, the cells that metabolize styrene in the lung differ in their number, location, and specificity among mice, rats, and humans.

Physiologically Based Pharmacokinetic (PBPK) modeling has shown that the target tissue concentration of SO is primarily due to localized tissue metabolism of styrene (Sarangapani *et al*., 2002). This model also predicted that at a given airborne concentration of styrene, the level of total SO in mouse terminal bronchioles is approximately 10 times higher than in rats and 100 times higher than in humans (Sarangapani *et al*., 2002). Evidence that local metabolism of styrene is responsible for the lung toxicity

of styrene is given by the fact that circulating levels of SO do not correlate with lung tumor incidence. Blood levels of SO were much higher in rats at non-tumorigenic concentrations than in mice at levels associated with an increased incidence of lung tumors (Cruzan *et al*., 1998; 2001). In addition, metabolism and cytotoxicity occur in the mouse lung after oral exposure to styrene (Green *et al*., 2001a), indicating that systemically-absorbed concentrations of styrene are preferentially metabolized in the mouse lung.

Studies in mice using a cyp2f2 inhibitor have shown that metabolism of styrene by cyp2f2 is necessary to cause lung toxicity (Green *et al*., 2001a,b). Inhibition of cyp2f2 also inhibited the cytotoxicity of 4-VP (Carlson, 2002), which is 10 times as toxic to the mouse lung as styrene and 5 times as toxic as SO (Carlson *et al*., 2002). Because 4-VP can be metabolized by cyp2f2 into further ring-oxidized metabolites of styrene (Carlson *et al*., 2001), this indicates that there is a subsequent metabolite of 4-VP that is responsible for lung cytotoxicity in mice. As mentioned above, the ring oxidation pathway of styrene metabolism is much more predominant in mice than in rats or humans, and it has been shown that intraperitoneal administration of 4-VP induced cytotoxicity in the terminal bronchioles of mice, but not rats (Cruzan *et al*., 2005).

The NTP draft profile for styrene states that a proposed mechanism for carcinogenicity of styrene is: "metabolic conversion of styrene to styrene-7,8-oxide and subsequent induction of DNA damage in the target tissue" (NTP, 2009). Extensive data show, however, that this suggestion of a genotoxic mode of action for styrene is unlikely.

Although SO can adduct to proteins and DNA, very low levels of SO DNA adducts have been observed *in vivo*. No increase in SO DNA adducts has been observed in mouse *vs*. rat lung or mouse lung *vs*. mouse liver after inhalation exposure to styrene; thus, the increased incidence of lung tumors in mice is not accompanied by an increase in DNA adducts (Boogaard *et al*., 2000; Cruzan *et al*., 2002).

The genotoxicity data for styrene are inconsistent (as reviewed by IARC, 2002). The mutagenicity data have been negative or weakly positive. There have been some positive results from *in vitro* assays for chromosomal effects, but *in vivo* studies do not indicate increases in chromosomal aberrations or micronuclei. A small increase in sister chromatid exchanges (SCEs) has been observed in animal studies of styrene, but in humans there has been no observed increase in SCEs in a dose-responsive manner. In a study by Vodicka *et al*. (2004), there was no clear association between styrene

exposure and chromosomal aberrations, micronuclei, single strand breaks, and DNA repair in styrene-exposed workers.

Although SO is directly genotoxic *in vitro*, oral administration of SO to mice and rats did not lead to systemic tumors, despite producing systemic concentrations of SO that were equal to or higher than those resulting from inhalation exposures (Sarangapani *et al*., 2002). In both species, tumors were only observed at the site of contact, the forestomach (Conti *et al*., 1988; Lijinsky, 1986; Ponomarkov *et al*., 1984). In these studies, oral exposure to SO also induced cell damage, repair, and increased proliferation, suggesting that the mechanism for tumor formation by SO in the forestomach is attributable to the increased cell proliferation resulting from the cellular damage induced by SO. This type of non-genotoxic mechanism of action may also explain the increased lung tumor incidence in mice after chronic exposure to styrene.

A non-genotoxic mode of action for styrene-induced lung tumors in mice is the most plausible mechanism for styrene carcinogenicity. Increased tumor incidence caused by styrene exposure has only been observed in one species and at one tissue site. The tumors were mostly benign and were observed late in life, causing no early mortality. Tumors were accompanied by organ toxicity and cell turnover, in the form of Clara cell damage and proliferation. Target organ metabolism of styrene is necessary for the observed toxicity and tumor formation, as oral administration of the genotoxic primary metabolite, SO, does not induce systemic tumors, and circulating levels of SO are not associated with increased tumor incidence. The genotoxicity data for styrene are inconsistent, and DNA adducts are observed at low levels after exposure and are not associated with tumor incidence.

The mechanistic evidence that suggests a specific, non-genotoxic mode of action for styrene in the responding animals is of questionable applicability to other animals, other tissues, and to humans. Styrene-induced cytotoxicity occurs in organs with high levels of the CYP2F family. Mouse lungs have a larger fraction of Cyp2f2-containing Clara cells than rat or human lungs, and the metabolism rates of styrene by the CYP2F family are higher in mice than in rats and are virtually non-existent in humans. In addition, styrene metabolites are removed more rapidly in rat and human tissues compared to mouse tissues (Green *et al*., 2001b). Consistent with these data, no styrene-induced toxicity, hyperplasia, or tumors have been observed in rat or human lungs. Because the levels of CYP2F enzymes are higher in rat lungs compared to human lungs, the lack of lung toxicity or tumor formation in rats makes it very

unlikely that a chemical that causes lung tumors by this mode of action in mice, but not rats, will cause human lung tumors.

In summary, styrene-induced lung tumors in mice arise from the lung-specific toxicity of styrene. This toxicity depends upon the localized metabolism of styrene to cytotoxic metabolites by mouse cyp2f2, which differs in both its specificity and rate of metabolism compared to rats and humans. In addition, the styrene metabolite believed to be responsible for lung toxicity in mice is rarely formed in humans. In accordance with this, epidemiological data do not show an increased incidence of lung cancer, or any cancer type, in humans exposed to styrene. These data will be discussed further in the next section.

5 Human data do not consistently show increased mortality or incidence of cancer

The NTP draft profile for styrene suggests that epidemiologic studies of workers exposed to styrene show increased mortality or incidence of lymphohematopoietic cancer and that some studies in the reinforced plastics industry provide suggestive evidence for increased incidences or mortality due to pancreatic and esophageal tumors (NTP, 2009). When considering the epidemiology evidence as a whole, however, there are no consistent associations between styrene exposure and any specific cancer type either within or among studies.

According to the Draft Background Document (NTP, 2008), the major epidemiology studies of styrene focus on 10 cohorts from the reinforced plastics and composites (RPC), styrene-butadiene latex rubber (SBR) and styrene/polystyrene (PS) industries, an occupational cohort in Finland reporting urinary concentrations of a styrene metabolite (Anttila *et al.*, 1998), and a cohort of students who attended high school adjacent to facilities that produced synthetic styrene-butadiene (Loughlin *et al.*, 1999; NTP, 2008). The highest exposures occurred in the RPC industry, followed by the SBR industry, and then the PS industry (NTP, 2008). Workers in the SBR industry were co-exposed to 1,3-butadiene, an established carcinogen. Although some studies adjusted for these co-exposures in some analyses, it has still been suggested that these chemicals may be responsible for any observed associations with styrene. As noted by Boffetta *et al*. (2008): "The excess leukemia mortality in the SBR industry is in line with what would be expected from exposure to the established carcinogen, 1,3-butadiene…with no evidence for an amplified effect from the co-exposure to styrene."

In certain studies, there were some statistically significant associations noted with some styrene exposure metrics for lymphohematopoietic, esophageal, and pancreatic cancers, but the risk estimates were not markedly large (*i.e.*, most were below 2 or 3) and were far outnumbered by null associations for each cancer type. In addition, most analyses were based on a small number of observed cases, which resulted in unstable estimates, vis-à-vis wide confidence intervals that either included or were generally close to 1. Furthermore, there were significant and non-significant negative associations reported for certain cancer types that were often as strong as positive associations reported for others. Just as it is unlikely that these negative associations are reflective of a protective mechanism for styrene, the few positive associations are unlikely to reflect a causal association.

If styrene were associated with cancer, then one would expect an exposure-response relationship within studies and among industries. Studies of RPC workers should carry the greatest weight in an assessment of the epidemiology data because these workers have the highest exposures, even though these cohorts are comprised of many short-term workers. Studies of SBR workers should carry less weight because the exposures are far lower, and 1,3-butadiene, even when adjusted for, cannot be completely ruled out as a confounder. Stronger associations between exposure and cancer risk were not observed in RPC workers. In addition, there was no indication of an increased risk with increased exposure within studies. Thus, when weighing these studies accordingly, the evidence for a lack of an effect becomes even stronger.

Although the NTP (2009) draft profile for styrene suggests increased risks for lymphohematopoietic cancers, each of these cancer types is unique, with an independent mode of action (Schottenfeld and Fraumeni, 2006), and there were no consistent associations with any specific lymphohematopoietic cancer either within or across studies. Risks of other types of cancer, such as pancreatic and esophageal cancer, were also not consistently observed among studies. Taken together, the evidence does not support the draft profile's suggestion that styrene exposure is associated with increased mortality or incidence of lymphohematopoietic, pancreatic, or esophogeal cancer and does not meet the NTP criteria for "limited" evidence of carcinogenicity in humans.

6 There is no concordance among human, experimental animal, and mode-of-action data

The NTP draft profile for styrene states that: "Although quantitative differences in styrene disposition exist across species, there are no demonstrated qualitative differences between humans and laboratory animals that contradict the relevance of the rodent cancer studies for evaluations of human hazard. The detection of styrene-7,8-oxide-DNA adducts at base-pairing sites and chromosomal aberrations in lymphocytes of styrene-exposed workers supports the potential human cancer hazard from styrene through a genotoxic mode-of-action" (NTP, 2009). This statement is incorrect for several reasons, as described below.

There is no concordance among the human, rodent, and mode-of-action data on the effects of styrene. The styrene-induced lung toxicity and tumor formation observed in mice are species-specific, as they are not observed in rats or humans exposed to styrene. The mechanistic data strongly suggests that these tumors are the result of mouse-specific lung toxicity that depends upon the localized metabolism of styrene by mouse cyp2f2. In humans, CYP2F2 does not appear to metabolize styrene. In addition, the metabolic pathway resulting in the formation of 4-VP, which may be the substrate for the cyp2f2-dependent cytotoxic metabolite in mouse lung, is a very minor pathway in humans compared to mice. There is a good deal of evidence that the styrene oxide mutagenicity, cited by the NTP draft profile as the basis for applying the mouse results to human risk projection, is not an important factor *in vivo* even in animals, is not consistent with the localization of mouse tumors, and in fact is not responsible for the mouse tumor response, so the ability of humans to form styrene oxide is not germane.

The epidemiological data as a whole do not suggest that styrene exposure is associated with any specific cancer type in humans, either within or among studies. The NTP draft profile, however, interprets these data as suggesting that styrene exposure increases the incidence of lymphohematopoietic cancers, and possibly pancreatic and esophageal cancers, in humans (NTP, 2009). Even if one accepts this interpretation, there have been no corresponding responses observed in animals, as no increased incidences of lymphohematopoietic, pancreatic, or esophageal tumors have been reported in styrene-exposed animals. The animal data also indicate that orally-administered styrene does not induce tumors systemically; although the data are unclear, in some studies an increased incidence of tumors was observed specifically in the mouse lung.

The studies in which SO-DNA adducts and chromosomal aberrations were detected in styrene-exposed workers do not support a carcinogenic role for styrene. These studies are limited by their small size and lack of controlling for potential confounders. In addition, styrene-exposed rodents form similar DNA adducts, with higher levels observed in rats than in mice, suggesting that these adducts are not associated with an increased incidence of tumors (Boogaard *et al.*, 2000; Cruzan *et al.*, 2002). Furthermore, agents that are known or expected to cause lymphohematopoietic cancers in humans are believed to act through immune dysregulation and not through DNA damage (Alexander *et al.*, 2007).

A genotoxic mode of action for styrene, either in animals or in humans, is not plausible. The genotoxicity data for styrene are inconsistent. Styrene is not carcinogenic in humans, and the animal and mechanistic data support a non-genotoxic mode of action for styrene. Styrene exposure has been associated with an increased tumor incidence in only one species and one site, mouse lung. The late onset and mostly benign lung tumors observed in mice were accompanied by lung cytotoxicity and cell proliferation and were dependent upon lung-specific metabolism of styrene. These data suggest a non-genotoxic mechanism of action attributable to increased cell proliferation in the lung resulting from the cellular damage induced upon styrene metabolism.

Taken together, the human, animal, and mode-of-action data for the effects of styrene do not support a conclusion of human carcinogenicity for styrene. The epidemiology data do not suggest that styrene exposure is associated with an increased incidence of any tumor type. The lack of correspondence of tumor incidence and tumor type among rodents, even within the same species, and humans indicates that there has been no particular type of cancer consistently observed among all available studies and renders the argument for the human carcinogenicity of styrene as implausible. The various data indicate that the only plausible mechanism for styrene-induced carcinogenesis is a non-genotoxic mode of action that is specific to the mouse lung. Thus, there is no evidence in the scientific literature that adequately supports the classification of styrene as a human carcinogen.

7 Conclusion

The NTP (2009) classification of styrene as "reasonably anticipated to be a human carcinogen" based on "limited" evidence of carcinogenicity in humans, "sufficient" evidence of carcinogenicity in animals, and supporting mechanistic data is not appropriate, given that the available data do not meet these criteria.

When the epidemiology evidence is considered as a whole, there are no consistent associations between styrene exposure and mortality or incidence of any cancer type, either within or among studies. These data show low numbers of observed cases, a lack of an exposure-response relationship, and concerns regarding co-exposures to known carcinogens and confounding. Thus, a causal interpretation is not credible, and the standards of "limited" evidence are not met.

The evidence for styrene-induced carcinogenesis in experimental animals does not meet the standards of "sufficient" evidence. Increased incidences of mostly benign tumors have been observed in certain strains of only one species (mice) and at one tissue site (lung). This tumor type is common in mice, and the tumors developed late in life in the presence of chronic cytotoxicity.

The mechanistic data on styrene suggest a non-genotoxic mode of action for styrene that is based on local cytoxicity and subsequent cell proliferation and is highly species-specific. Thus, this mode of action is not applicable to humans or other animals and does not support the classification of styrene as a human carcinogen.

The rationale for using rodent bioassay results as indicators of possible human carcinogenicity rests on the broad similarity among mammals in anatomy, physiology, and biochemistry; the applicability of a rodent response as an indicator of potential human risk amounts to hypothesizing that, owing to this underlying commonality, the carcinogenic processes responsible for the animal results could also plausibly occur in humans. That is, one is hypothetically *generalizing* the phenomenon from the particular animal species showing the response to other mammals, including humans.

For styrene, however, it is clear that the processes responsible for the tumorigenesis observed in mice do not occur in rats. It is not only that rats do not show a tumor impact of styrene inhalation (the hypothesized generality of which across mammals is the basis for inferring its relevance to humans), but also that the specific mode of action – the tissue-specific metabolic activation – is not present. In short, the proposed generalization of effects across mammals is contradicted. Moreover, there is no indication that the mode of action would be present in humans, either.

I have argued that the animal evidence is not "sufficient" according to NTP's standards of interpretation. Beyond this, however, it is clear that the animal evidence for a carcinogenic effect of styrene applies only to mice and not to rats, so any "sufficiency" of animal evidence does not apply to all

animals – and to the degree that it doesn't, its applicability to humans is also questionable, since one would have to propose why, against all available evidence, humans should be supposed to be like the mice and not like the rats in their response to styrene.

I have also argued that the human studies on styrene and cancer do not support a conclusion of "limited" evidence. There are no consistent responses among the human data of the kind that one would expect if there were true biological causation. The diversity of proposed tumor endpoints in human studies raises more questions than it answers – why is it that styrene would affect some tumor responses in some studies, and other responses (requiring other modes of action) in other studies? If there were truly a mode of action of sufficient generality and broadness to cause such a variety of tumor responses in humans, why is there no sign of it – and why is there no indication of haematopoietic cancers – in rats or in mice?

To bring together animal, human, and mode-of-action data into an overall weight of evidence conclusion about the potential for human carcinogenicity, one seeks to characterize the likelihood of a common thread that ties together the evidence from the different sources and proposes a biologically plausible line of reasoning as to why a potential hazard in humans is indicated. For styrene, there is no such commonality. The mouse tumor responses are best interpreted as a species-specific phenomenon that does not apply to rats and for which there is evidence against its applicability to humans. There is no consistent response among human studies, and the hypothesized human effects have no counterpart in the animal data. No mode of carcinogenic action has been identified that would apply beyond the mice. All together, these data do not support the NTP draft profile's characterization of styrene as "reasonably anticipated to be a human carcinogen," but rather a characterization of "not classifiable."

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